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(21) International Application Number: PCT/GB93/00932 (22) International Filing Date: 6 May 1993 (06.05.93) (30) Priority data: 9209860.7 7 May 1992 (07.05.92) GB (71) Applicant (for AU CA GB IE only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB). (71) Applicant (for all designated States except AU CA GB IE): UNILEVER NV [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL). (72) Inventors: CRAWFORD, Duncan, John, Knox ; 46 Reynolds Road, Shirley, Southampton SO1 5GS (GB). RAWLINGS, Anthony, Vincent ; 509 Spencer Drive, Wyckoff, NJ 07481 (US). SCOTT, Ian, Richard ; 69 Arcadia Road, Allendale, NJ 07401 (US).		(74) Agent: TONGE, R., J.; Patent Division, Unilever plc, Colworth House, Sharnbrook, Bedford MK44 1LQ (GB). (81) Designated States: AU, BR, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.	
(54) Title: SYNTHESIS OF COSMETIC INGREDIENT			
<div style="text-align: center;"> $\begin{array}{c} \text{Y} - \text{O} - (\text{C}_8\text{H}_b) - \overset{\text{O}}{\parallel} \text{C} - \text{NH} \\ \\ \text{CH} - \text{CH}_2\text{OH} \\ \\ \text{CH}_3 - (\text{CH}_2)_n - \text{A} - \text{CHOH} \end{array} \quad (1)$ </div> <div style="text-align: center;"> $\begin{array}{c} \text{O} \\ \parallel \\ - \text{C} - (\text{C}_x\text{H}_y\text{Z}_z) \text{CH}_3 \end{array} \quad (2)$ </div>			
(57) Abstract <p>A method of synthesis of ω-hydroxy fatty acid containing ceramides having general structure (1), where A represents CH_2 or $-\text{CH} = \text{CH}-$, Y represents a residue of a C_{14} to C_{22} fatty acid having structure (2) (where Z is $-\text{OH}$ or an epoxy oxygen, x is an integer of from 12 to 20, y is an integer of from 20 to 40, z is 0 or an integer of from 1 to 4, a is an integer of from 8 to 50, b is an integer of from 10 to 100), and n is an integer of from 7 to 27.</p>			

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SYNTHESIS OF COSMETIC INGREDIENTFIELD OF THE INVENTION

5 The invention relates to a method of synthesis of ω -hydroxy fatty acid containing ceramides.

BACKGROUND AND PRIOR ART

10 It is well established that ceramides have a vital role in the production and maintenance of the water permeability barrier of the skin. Ceramides, or substances closely related to them, have been widely disclosed as components of skin care compositions.

15 In particular EP 0 097 059 (Unilever) discloses the vital role played by ω -linoleoyl ceramides in the water barrier of the skin and describes the application for skin care of such ω -linoleoyl ceramides.

20 It is known in the art that skin ceramides (including ω -hydroxy fatty acid containing ceramides) may be extracted from mammalian stratum corneum cells.

25 It has been proposed for example in US 3,660,566 (Lever Brothers Company), to extract animal stratum corneum from epidermis and then separate the stratum corneum into a lipid-rich fraction and a cellular fraction, and then to employ the lipid-rich fraction in cosmetic compositions.

30 It is also proposed in GB 2 178 312 (Kao Corporation) to extract lipids from horny cells of mammals such as the pig, and then to employ the lipid so extracted in cosmetic compositions which are useful for treating dry skin
35 conditions in man.

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However, to extract ω -hydroxy fatty acid containing ceramides in high enough levels for incorporation into skin care products causes problems. Furthermore it is undesirable for the source of the cosmetic ingredient to be from animals.

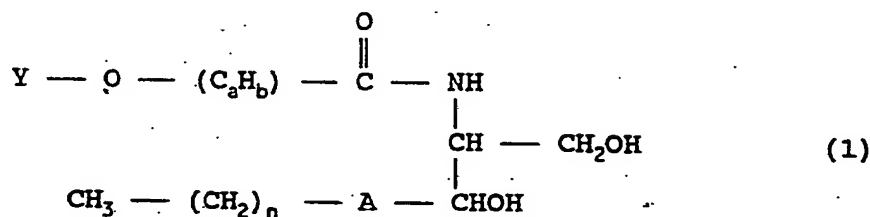
Synthetic Pseudo-ceramides have also been widely disclosed in the literature, for example by Kao Corporation in EP 0 227 994 which discloses synthetic analogues of ceramides which have some similar properties to natural ceramides but which are relatively cheaper to produce. However, the degree of skin benefit attributable to such analogues is limited to the extent that they do not fully mimic the natural ceramides of the skin.

It is therefore desirable to be able to synthesise naturally-occurring ω -hydroxy fatty acid containing ceramides using a synthesis route, which is not too expensive, to allow the possibility of the synthesised ceramides being incorporated at functional levels into cosmetic compositions.

DEFINITION OF THE INVENTION

Accordingly, the invention provides:

A method of synthesising an ω -hydroxy fatty acid containing ceramide having the general structure (1):

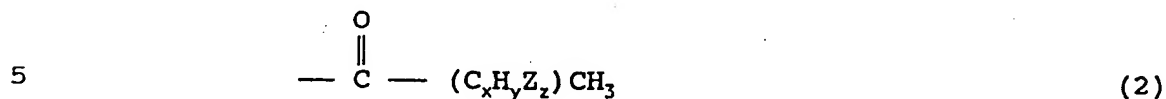


where A represents CH_2 or $-\text{CH}=\text{CH}-$

Y represents a residue of a C_{14} to C_{22} fatty acid having the

- 3 -

structure (2)--



where Z is - OH or an epoxy oxygen

x is an integer of from 12 to 20

y is an integer of from 20 to 40

10 z is 0 or an integer of from 1 to 4

a is an integer of from 8 to 50

b is an integer of from 10 to 100

and n is an integer of from 7 to 27

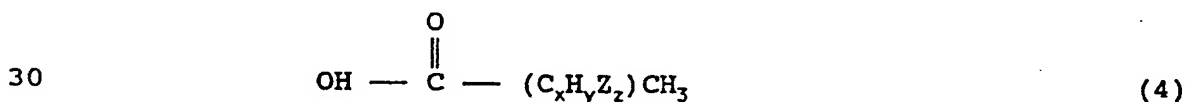
15 selected from synthesis A, synthesis B, synthesis C and synthesis D wherein;

synthesis A comprises;

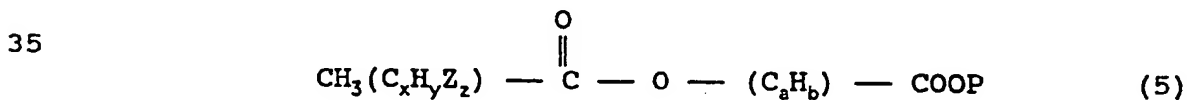
20 (ia) coupling an ω -hydroxy fatty acid with a protected carboxyl group having the general structure (3);



25 where P is a protection group with a C_{14-22} fatty acid having the general structure (4);

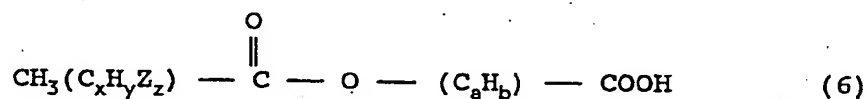


to give an intermediate having the general structure (5);



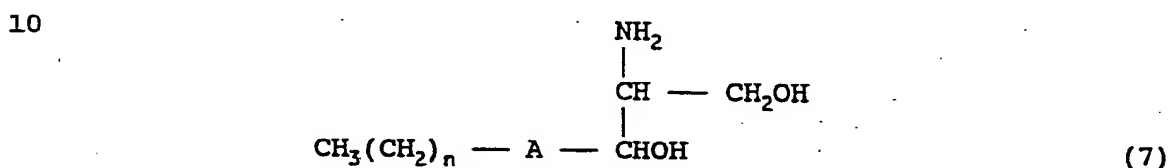
(iia) deprotection of the intermediate (5) to provide an intermediate having the general structure (6);

- 4 -



5 ; and

(iiia) coupling the intermediate (6) with sphingosine having the general structure (7);



15

to form the ω -hydroxy fatty acid containing ceramide having the general structure (1);

synthesis B comprises;

20

(ib) converting a terminal acetylenic alcohol having the general structure (8);



25

into an intermediate having the general structure (9);



30

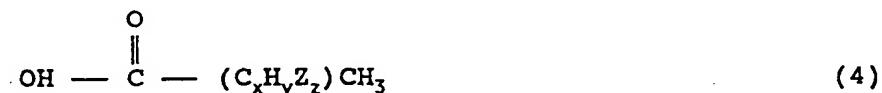
then converting this intermediate into an intermediate having the general structure (10);



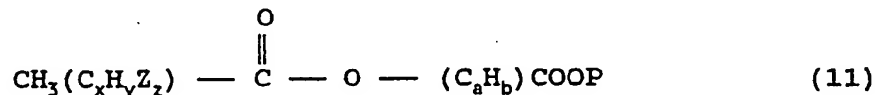
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(iib) coupling the intermediate (10) with a C_{14-22} fatty acid having the general structure (4);

- 5 -

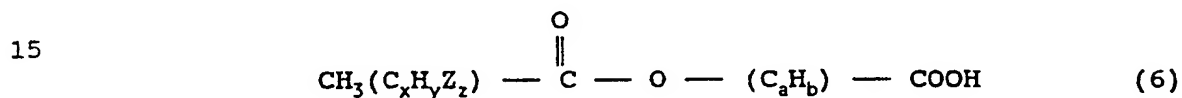


5 to give an intermediate having the general structure (11);



10

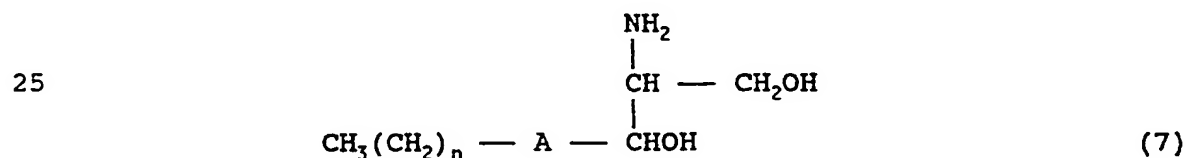
then selectively converting the intermediate (11) into an intermediate having the general structure (6);



15

; and

20 (iiib) coupling the intermediate (6) with sphingosine having the general structure (7);



25

30 to form the ω -hydroxy fatty acid containing ceramide having the general structure (1);

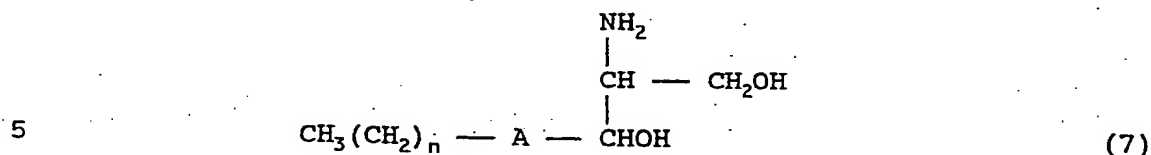
synthesis C comprises:

35 (ic) coupling an ω -hydroxy fatty acid having the general structure (12);

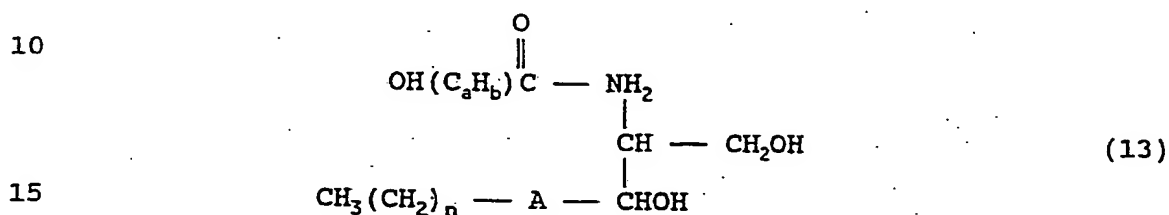


40 with a sphingosine having the general structure (7);

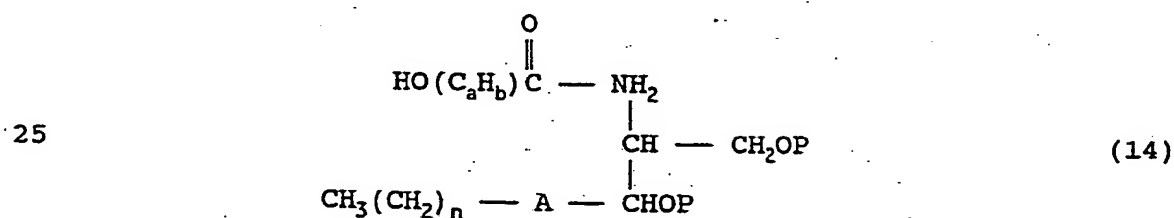
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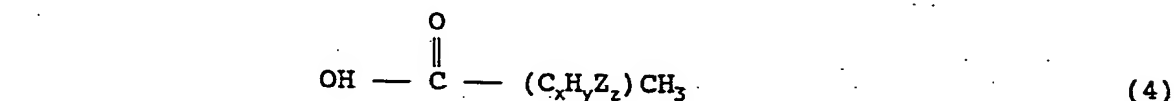
to form an intermediate having the general structure (13);



(iic) protection of the hydroxyl groups of the intermediate having the general structure (13) to give an intermediate having the general structure (14);



(iiic) esterification of the intermediate (14) with a C_{14} to C_{22} fatty acid having the general structure (4);



; and

(ivc) removal of the protection groups to provide the ω -hydroxy fatty acid containing ceramide having the general structure (1); and

synthesis D comprises:

- 7 -

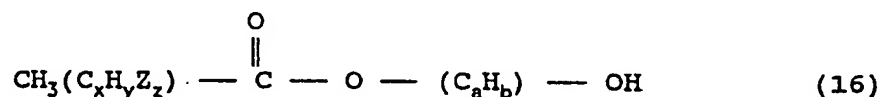
(id) coupling a long chain diol having the general structure (15);



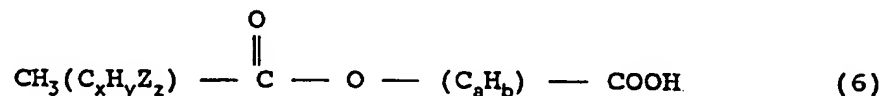
with a C_{14-22} fatty acid having the general structure (4);



to give an intermediate having the general structure (16);

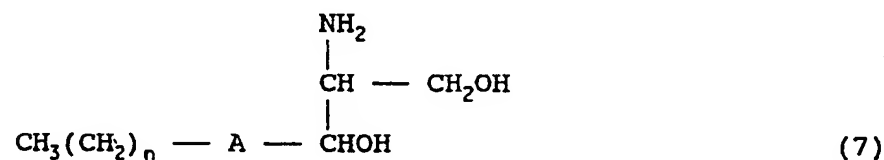


(iia) oxidation of the free hydroxyl group on intermediate (16) to give an intermediate having the general structure (6);



; and

(iiia) coupling the intermediate (6) with sphingosine having the general structure (7);



to form the ω -hydroxy fatty acid containing ceramide having the general structure (1).

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DISCLOSURE OF THE INVENTION

5 The invention concerns the synthesis of ω -hydroxy fatty acid containing ceramides having the general structure (1) given above.

10 With reference to structure (1) the value of a is preferably an integer of from 20 to 30 and the value of b is most preferably an integer of from 40 to 60.

15 Also with reference to structure (1) the group Y preferably represents a straight chain saturated C_{16-18} fatty acid residue or a straight chain all cis n-6,9 di-unsaturated C_{16-18} fatty acid residue, more preferably represents a fatty acid selected from oleic acid, palmitic acid, stearic acid and linoleic acid, and is most preferably a linoleic acid residue.

20 Also with reference to structures (1) or (7), n is preferably 12.

Also with reference to structures (1) or (7), A is preferably the group $—CH=CH—$.

25 With reference to synthesis A, B and C, the protection group (P) is preferably t-butylalcohol.

30 Important in the synthesis of the ceramides having the general structure (1) is the order in which the component parts (sphingosine, ω -hydroxy fatty acid and C_{14-22} fatty acid) are coupled together. There are four alternative synthesis routes (ie synthesis A, B, C and D).

35 Synthesis A comprises;

(ia) coupling a ω -hydroxy fatty acid with a protected carboxyl group having the general structure (3);

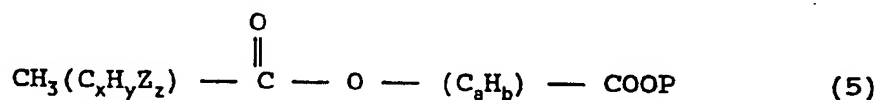
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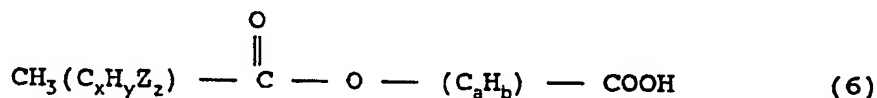
where P is a protection group
with a C_{14-22} fatty acid having the general structure (4);



under standard esterification reaction conditions, for example dicyclohexylcarbodiimide (DCC)/dimethylamino pyridine (DMAP), to give any intermediate having the general structure (5);

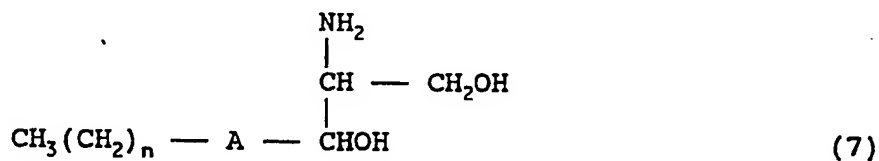


(iia) deprotection of the intermediate (5), preferably by treatment with toluenesulphonic acid in refluxing benzene to provide an intermediate having the general structure (6);



; and

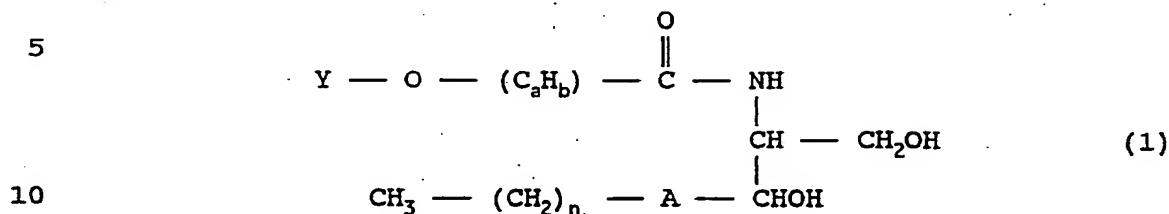
(iiia) coupling the intermediate (6) with sphingosine having the general structure (7);



under standard peptide bond coupling conditions (for example, DCC/DMAP in chloroform, 2 hours, 0°C) to form the

- 10 -

ω -hydroxy fatty acid containing ceramide having the general structure (1).



Synthesis B comprises;

(ib) converting a terminal acetylenic alcohol having the general structure (8);



into an intermediate having the general structure (9);



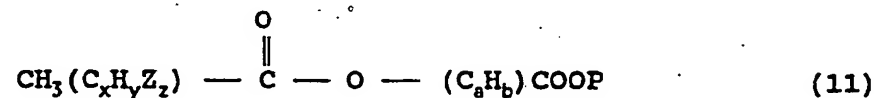
then converting this intermediate into an intermediate having the general structure (10);



(iib) coupling the intermediate (10) with a C_{14-22} fatty acid having the general structure (4);



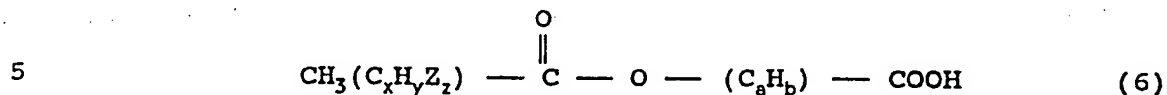
to give an intermediate having the general structure (11);



then selectively converting the intermediate (11) into an

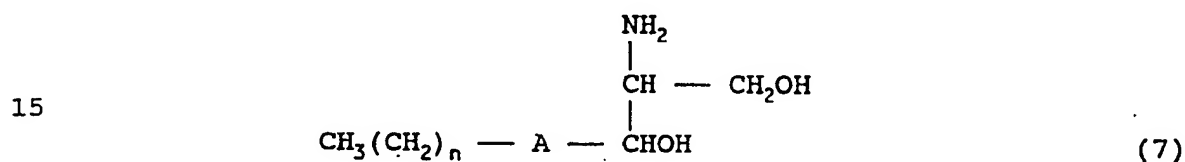
- 11 -

intermediate having the general structure (6);

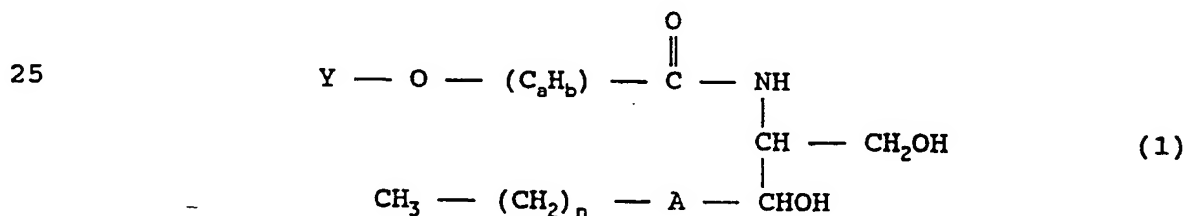


; and

(iiib) coupling the intermediate (6) with sphingosine having the general structure (7);



under standard peptide bond coupling conditions (for example, DCC/DMAP in chloroform, 2 hours, 0°C) to form the ω -hydroxy fatty acid containing ceramide having the general structure (1).



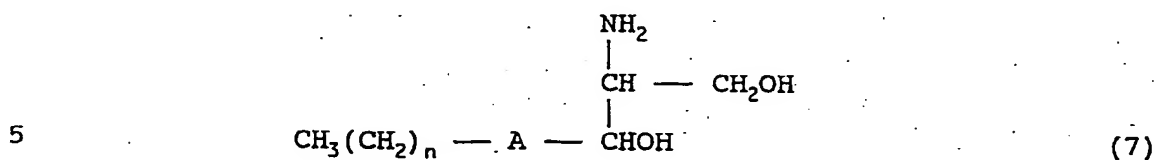
Synthesis C comprises;

(ic) coupling an ω -hydroxy fatty acid having the general structure (12);

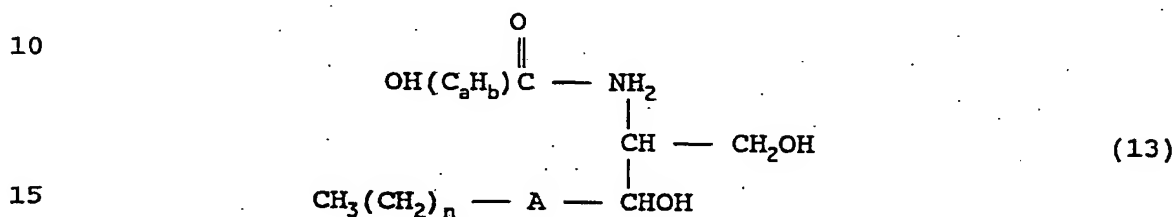


with a sphingosine having the general structure (7);

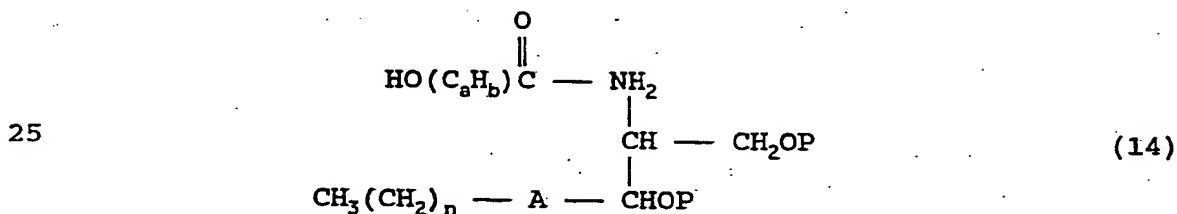
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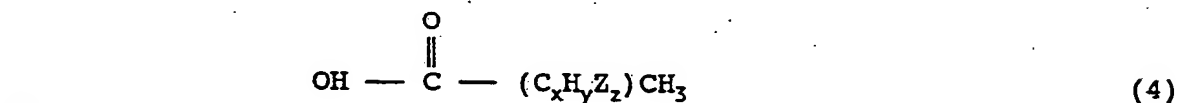
to form an intermediate having the general structure (13);



(iic) protection of the hydroxyl groups of the intermediate having the general structure (13) to give an intermediate having the general structure (14);



(iiic) esterification of the intermediate (14) with a C_{14} to C_{22} fatty acid having the general structure (4):



; and

(ivc) removal of the protection groups to provide the ω -hydroxy fatty acid containing ceramide having the general structure (1).

Synthesis D comprises:

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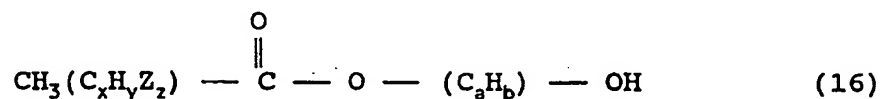
(id) coupling a long chain diol having the general structure (15):



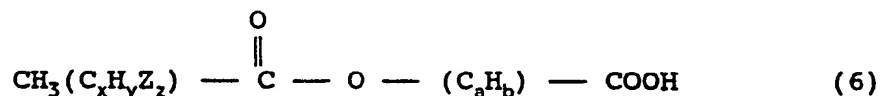
with a C_{14-22} fatty acid having the general structure (4);



to give an intermediate having the general structure (16);

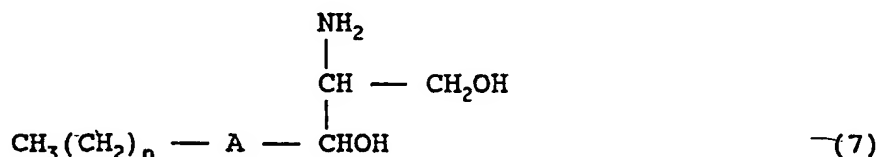


(iia) oxidation of the free hydroxyl group on intermediate (16) to give an intermediate having the general structure (6);



; and

(iiid) coupling the intermediate (6) with sphingosine having the general structure (7);

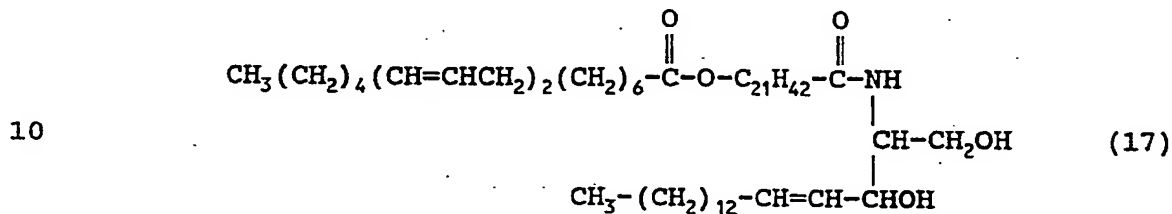


under standard peptide bond coupling conditions (for example DCC/DMAP in chloroform, 2 hours, 0°C) to form the ω -hydroxy fatty acid containing ceramide having the general structure (1).

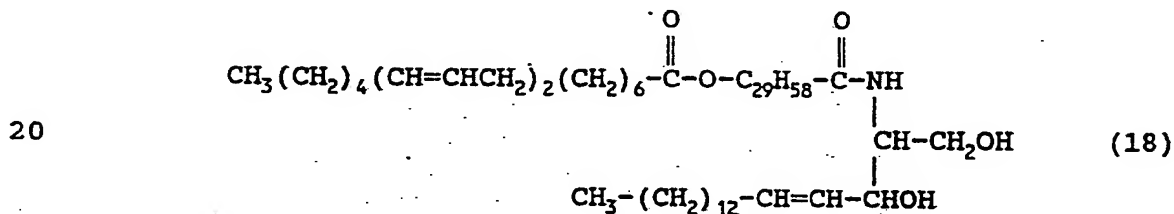
Synthesis routes A and D are preferred.

Specific examples of these synthesised ceramides are those having the structures (17) to (21):

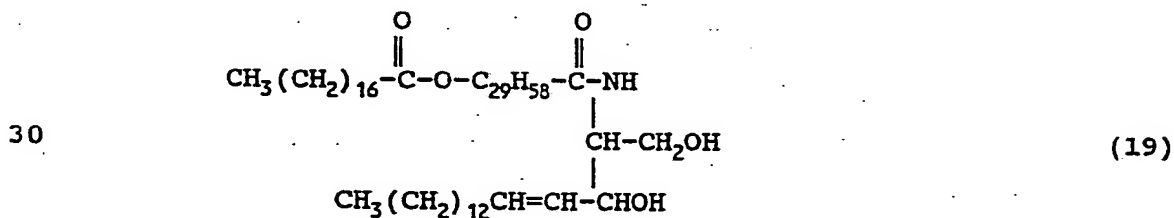
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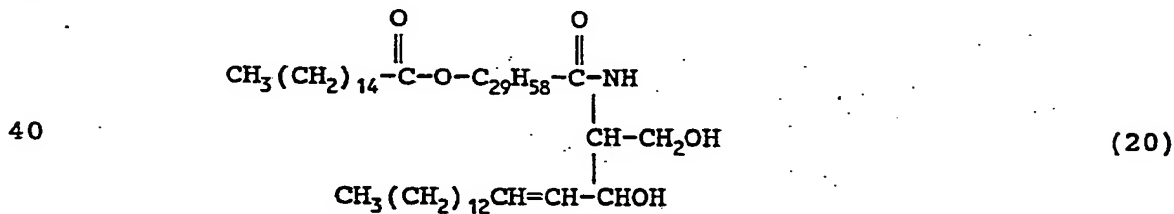
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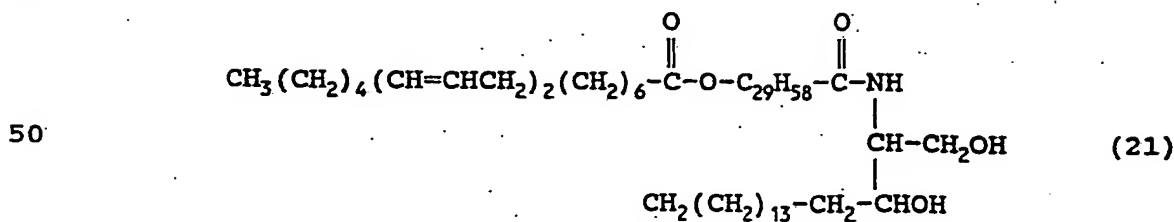
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- 15 -

The ω -hydroxy fatty acid

The ω -hydroxy fatty acid having the general structure (12);



may be obtained either by synthesis or by extraction from a natural source.

10 Any appropriate synthesis route for ω -hydroxy fatty acids may be used. An example of a suitable synthesis route is disclosed by Abrams (1981) in Chemistry and Physics of Lipids 28, 379 which describes the synthesis of ω -hydroxydocosanoic acid.

15 The synthesis of ω -hydroxy fatty acids is based upon the "acetylenic zipper" reaction in which potassium or sodium aminopropylamine effects the isomerization of an internal triple bond of an alkyne or alkyn-1-ol exclusively to the
20 terminal alkyne. Jones oxidation, esterification, hydroboration and oxidation yielded the ω -hydroxy fatty acid and/or ester.

ω -hydroxy fatty acids may be isolated from plants, beeswax,
25 or woolwax.

 The required ω -hydroxy fatty acid is preferably synthesised.

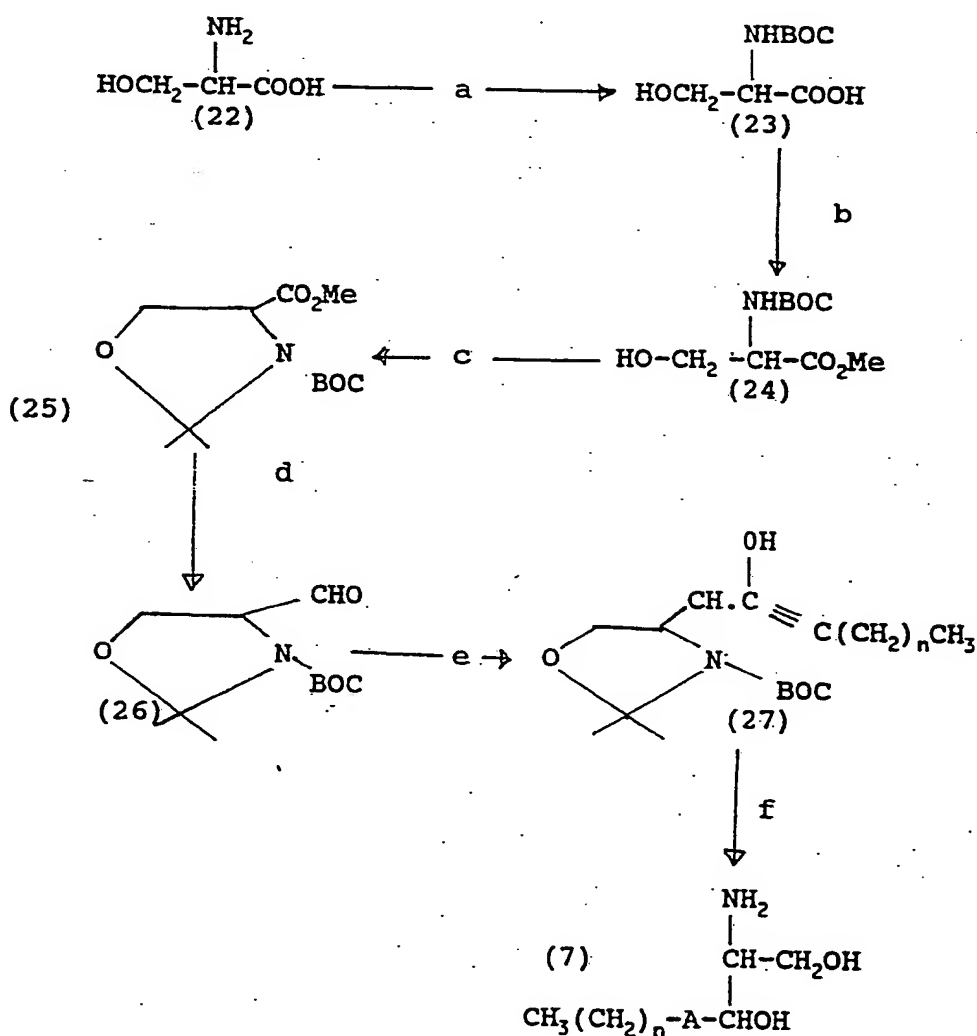
30 Sphingosine

 Synthesis of sphingosine has been disclosed by Garner et al (1988) J Org Chem 53 4396; Garner et al (1987) J Org Chem 52 2361 and Herald et al (1988) Helvetica Chimica Acta 71
35 354.

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Sphingosine was shown to be synthesised from either commercially available N-BOC-serine or from L-serine which had been treated with di-tert-butyl dicarbonate followed by esterification with diazomethane. The oxazolidine was formed under acid-catalysed conditions and the free aldehyde was reacted with lithium-1-pentadecyne to derive propargylic alcohols which were reduced to sphingosine.

However, the following novel synthesis route shown in Scheme 1 is preferred to any previously disclosed synthesis route.

Scheme 1

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Reagents

- a. (BOC)₂O, aqueous NaOH
- b. CH₂N₂, ether
- 5 c. Dimethoxypropane, TsOH, benzene
- d. Di-iso-butylaluminium hydride, toluene
- e. CH₃(CH₂)_nC≡CLi, tetrahydrofuran,
Hexamethylphosphorus-triamide (HMPT)
- 10 f. Lithium, ethylamine

10 In order to obtain the molecule with the correct stereochemistry, the cheap and readily available L-serine (22) was used as a starting material, effectively establishing the correct configuration for position 2 on the sphingosine chain. In the reaction sequence the amino group of serine was first protected with the tert-butoxycarbonyl (BOC) group (23), which is converted to the methyl ester (24) on treatment with ethereal diazomethane. 15 The remaining NH and OH groups on this molecule are then protected by conversion to the cyclic oxazolidine (25) on treatment with dimethoxypropane with a catalytic amount of toluene sulphonic acid in benzene, and the ester function reduced to the aldehyde (26) on treatment with di-iso-butylaluminium hydride (DI-BAL) in toluene. Treatment of 25 this aldehyde with CH₃(CH₂)_nC≡CLi in tetrahydrofuran (THF) in the presence of Hexamethylphosphorustriamide (HMPT) gave a mixture of erythro (27), and threo diastereoisomers. The erythro diastereoisomer (27) with the correct stereochemistry for D-erythro-sphingosine was obtained 30 following chromatography on silica gel. Treatment of (27) with lithium in ethylamine gave the target molecule D-erythro-sphingosine (7).

35 Note that by using different conditions it is possible to almost completely reverse the stereoselection in the addition of the lithium acetylide to the aldehyde (26). Thus this synthesis can be easily modified to produce any

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of the four stereoisomers of sphingosine with any given chain length. The naturally occurring D-erythro-stereoisomer is preferred.

5 Use Of Synthesised Ceramides (1) In Cosmetic Compositions

Ceramides synthesised in accordance with the invention can be incorporated into cosmetic compositions intended for topical application to human skin, hair, or nails in an
10 amount of from 0.0001 to 10%, preferably from 0.001 to 5% by weight of such compositions.

Topical application to human skin, hair or nails of such cosmetic compositions can improve the barrier and
15 elasticity properties of the epidermis of the skin, thus improving moisture retention and as a treatment of dry skin or detergent damaged skin.

EXAMPLES

20

The invention is illustrated by the following examples.

EXAMPLE 1

25 Synthesis of C₂₂ lineoyl ceramide 1 (17)

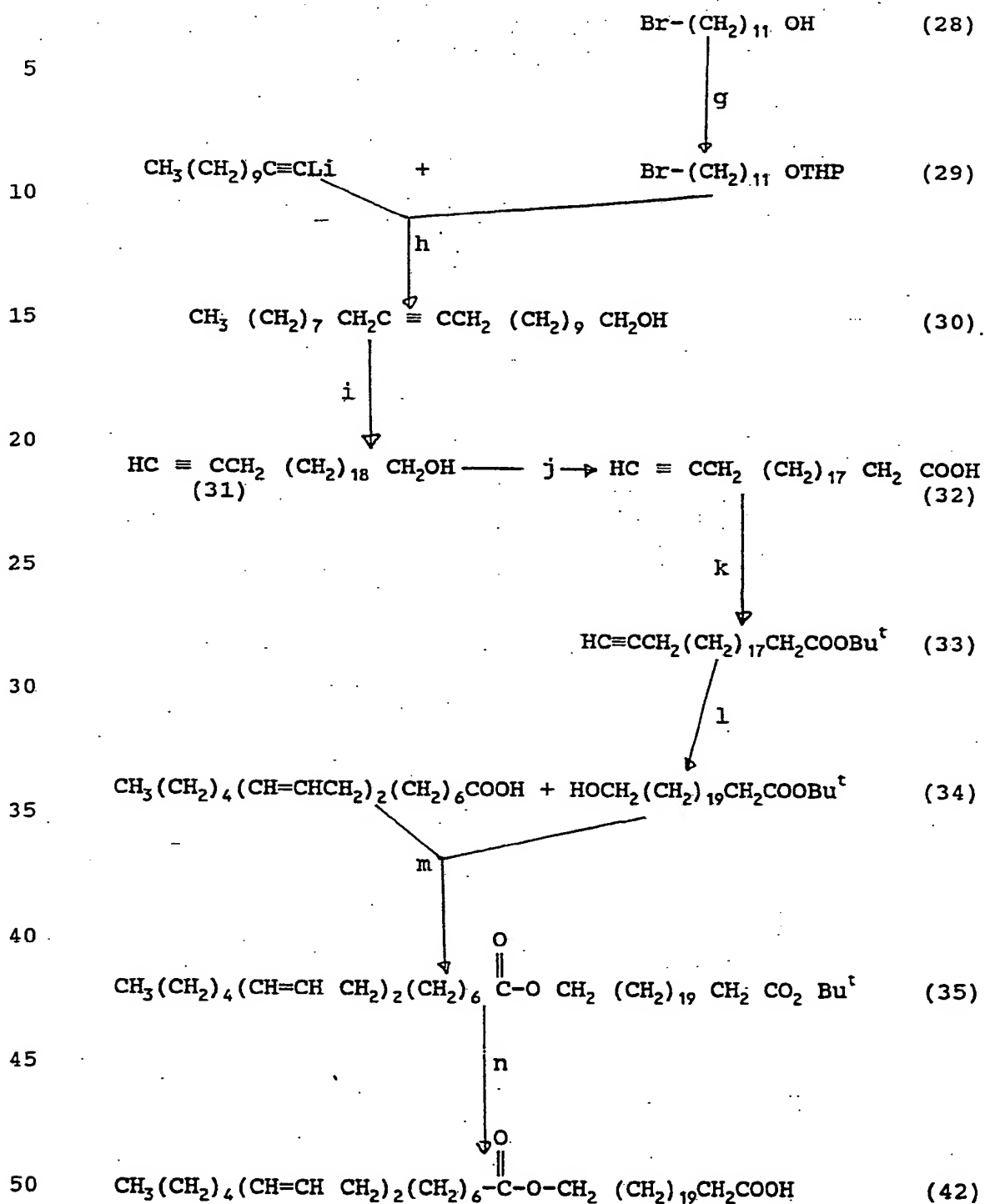
Synthesis of ω -hydroxy acid and coupling with linoleic acid to form 22-linoleoyloxydocosanic Acid (42)

30 The C₂₂ chain was put together as follows:- (see Scheme 2) commercially available 11-bromoundecanol (28) was protected as the THP ether by treatment with dihydropyran in the presence of toluenesulphonic acid to give the compound (29) in 99% yield. Alkylation of this material with 1-
35 Lithioundecyne in dimethoxyethane gave the product with C₂₂ chain length (30) in 80% yield as a crystalline solid. This material was converted to the terminal acetylenic

- 19 -

alcohol (31) on treatment with strongly basic conditions (acetylene zip reaction). This material is extremely significant in our synthetic route as it contains the C₂₂ chain length and has functionality which can be easily manipulated at both ends. Conversion of (31) to the corresponding acetylenic acid (32) was achieved in 83% yield by the use of Jones reagent, and (32) was in turn converted to the tert-butyl ester (33) in 86% yield on treatment with Dicyclohexyl carbodimide (DCC), Dimethylamino pyridine (DMAP); Dimethylamino pyridine trifluoro acetic acid (DMAP.TFA) and tert-butyl alcohol in chloroform. The acetylenic function on (33) was converted to the required alcohol (34) on treatment with borane dimethyl sulphide complex followed by alkaline oxidation, thus giving the initial target of ω -hydroxydocosanic acid (34) with selective protection on the acid functionality. As expected, this compound was found to couple smoothly with linoleic acid under standard conditions (DCC/DMAP) to give the linoleic acid- ω -hydroxydocosanic acid fragment of ceramide protected as the tert-butyl ester (35). Deprotection of this material occurred smoothly in 73% yield on treatment with toluenesulphonic acid in refluxing benzene to give the required linoleic acid- ω -hydroxydocosanic acid fragment (42) as a white crystalline solid in good overall yield (15% from 11 - bromoundecanol).

Scheme 2



- 21 -

Reagents

- g dihydropyran, toluene sulphonic acid hydrate (T_sOH),
CH₂Cl₂
5 h Lithium amide, dimethoxyethane
i NaH, Diaminopropane
j Jones reagent, acetone
k DCC, DMAP, DMAP.TFA, tertiary butyl alcohol, CHCl₃
l Borane dimethyl sulphide complex, THF
10 m DCC, DMAP, CHCl₃
n TSOH, Benzene

Experimental For The Synthesis Of C₂₂ Linoleic Ceramide 115 11-Bromoundecanol THP ether (29)

A solution containing 11-bromoundecanol (50g, 0.199mol) and dihydropyran (20ml, 18.44g, 0.219mol) in dry, ethanol-free methylene chloride (333ml) at room temperature under an
20 atmosphere of nitrogen was treated with a catalytic amount of toluenesulphonic acid, and the resulting solution stirred for 10 minutes, after which time tlc indicated the reaction to be complete. Aqueous sodium bicarbonate was added, the organic layer separated, dried (MgSO₄), filtered
25 and evaporated. The crude material obtained in this manner was purified by chromatography on silica (500g). Elution with petrol followed by 1% ether/petrol gave the title compound (29) as a clear oil (66.19g, 99%).

30 Docos-12-ynol (30)

A solution of undec-1-yne (15.22g, 0.1mol) in dimethoxyethane (100ml) at room temperature under nitrogen was treated with lithium amide (2.30g, 0.1mol) and the
35 resulting solution heated at reflux for 4 hour, after which time a solution of 11-bromoundecanol THP ether (29) in DME (50ml) was added and reflux maintained over night, after

- 22 -

which time tlc indicated the reaction to be complete. The mixture was poured onto water and extracted with ether three times. The combined organics were dried (MgSO_4), filtered and evaporated to leave a red oil (approx 60g) which was dissolved in methanol (300ml), treated with a catalytic amount of toluene sulphonic acid and heated at reflux for 30 minutes. On cooling, flesh coloured crystals separated which were collected and washed with methanol to give docos-12-ynol (30) (23.61g, 80%) mp 38-39°C.

Docos-21-ynol (31)

Diaminopropane (DAP) (150ml) at room temperature under nitrogen was stirred and treated with sodium hydride (100%, 13.55g, 0.56mol). The resulting solution was then warmed to 70°C for 1 hour after which time further DAP (30ml) is added followed by docos-12-ynol (22g, 0.068mol). The resulting solution was then left at 70°C overnight, cooled and poured into water. The aqueous mixture was extracted three times with chloroform, the combined organic dried (MgSO_4), filtered and evaporated to leave a pinkish residue. Recrystallisation from methanol gave docos-21-ynol (31) (18.05g, 84%) as white crystals mp 64-70°C.

Docos-21-ynoic Acid (32)

Docos-21-ynol (12g, 0.037mol) was dissolved in warm acetone (600ml) treated with Jones reagent (20ml). After stirring for 30 minutes tlc showed complete reaction so the excess Jones reagent was quenched with propane-2-ol and the volatile components removed in vacuo. The organic fractions were combined, dried (MgSO_4), filtered and evaporated to leave docos-21-ynoic acid (32) as a pale green solid (10.4g, 83%).

- 23 -

Docos-21-ynoic Acid, Tert.-Butyl Ester (33)

The acid (32) (6.4g, 0.019mol) was dissolved in dry, ethanol free, chloroform (120ml) at room temperature under nitrogen and sequentially treated with dimethylaminopyridine (2.55g, 0.0209mol), tert butanol (14.09g, 0.19mol), dimethylaminopyridine-trifluoroacetic acid complex (4.94g, 0.0209mol) and dicyclohexylcarbodiimide (4.31g, 0.0209mol). The resulting solution was stirred at room temperature overnight then filtered, the organic layer washed with 5% acetic acid in water, then dried (MgSO_4), filtered and evaporated. The residue was purified by chromatography on silica gel (50g). Elution with 10% ether in petrol gave the title compound (33) as white crystals (6.45g, 86%) mp 44-45°C.

Tert.-Butyl 22-Hydroxydocosanoate (34)

A solution of the acetylene (33) (0.4g, 1.02mmol) in dry THF (5ml) at room temperature under nitrogen was treated with borane-dimethyl sulphide complex (1.04ml of a 2M solution in THF, 2.08mmol) and the resulting solution stirred for 1 hour, after which time no starting material was present on tlc. The reaction was then treated sequentially with water (2ml), 2N aqueous sodium hydroxide (1ml) and 30% hydrogen peroxide (1ml). The resulting solution was left a further 15 minutes, then ethyl acetate added and the organic layer separated. The aqueous layer was re-extracted with ethyl acetate, the organic layers combined, dried (MgSO_4) and evaporated. The crude product obtained in this manner was purified on silica (5g). Elution with 10% ethyl acetate in petrol gave tert.-butyl 22-hydroxydocosanoate (34) as a white solid (0.23g, 54%).

- 24 -

22-Linoleoyloxydocosanoic Acid, Tert.-Butyl Ester (35)

A solution containing tert.-butyl 22-hydroxydocosanoate (34) (2.17g, 5.26mmol) and linoleic acid (1.45g, 5.26mmol) in dry ethanol free chloroform (50ml) at room temperature under nitrogen was treated with dimethylaminopyridine (0.064g, 0.524mmol) and dicyclohexylcarbodiimide (1.19g, 5.78mmol), and the resulting solution was stirred for three hours at room temperature, after which time tlc indicated that the reaction is complete. The mixture was filtered and the filtrate washed with 5% acetic acid, then water, then dried (MgSO_4), filtered and evaporated. The residue was purified by chromatography on silica (30g). Elution with petrol followed by 5% ether in petrol gave the title compound (35) as a white wax (2.18g, 79%).

22-Linoleoyloxydocosanic Acid (42)

A solution of the ester (35) (0.15g, 0.222mmol) in dry benzene (3ml) under nitrogen was treated with a catalytic amount of toluenesulphonic acid and the resulting solution heated at reflux for 5 hours, after which time no starting material remained on tlc. The volatile components were removed in vacuo and the residue chromatographed on silica (5g). Elution with 10% ether in petrol gave the title compound (42) as a white wax (0.1g, 73%).

N-Boc-L-Serine (23)

L-Serine (10g, 0.95mol) was dissolved in 1N sodium hydroxide (195ml) and the solution stirred and cooled to 0°C. A solution of BOC anhydride (24.7g, 0.113mol) in dioxane (88ml) was then added and the resulting solution allowed to stir up to room temperature over 1.5 hours, after which time tlc showed the reaction to be complete. The solution was then reduced to half volume in vacuo and the pH adjusted to 3 by the addition of 1N potassium

- 25 -

bisulphate (195ml). The mixture was then extracted with ethyl acetate twice, the organic combined, dried (MgSO_4), filtered and evaporated to leave the product (23) as a sticky foam (18.54g, 96%).

5

N-Boc-L-Serine Methyl Ester (24)

A solution of the acid (23) in ether (200ml) at room temperature was treated with ethereal diazomethane until the yellow colour just persisted and tlc showed there to be no starting material present. The volatiles were then removed in vacuo leaving the product (24) as a light orange oil (17.89g, 90%).

10

3-(1,1-Dimethylethyl)-4-Methyl(S)-2,2-Dimethyl-3,4-Oxazolidinedicarboxylate (25)

A solution of N-Boc-L-serine ester (24) (17.89g, 0.0816mol) in benzene (285ml) was treated with dimethoxypropane (21ml, 0.17mol) and toluenesulphonic acid (0.2g, catalytic). The resulting solution was then heated at reflux under an atmosphere of nitrogen for 30 minutes, and the volatile components removed under reduced pressure. The residue was dissolved in ether, washed with aqueous sodium bicarbonate, dried (MgSO_4) filtered and evaporated. The residue was then distilled at reduced pressure to give the title compound (25) as a clear oil (17.24g, 81%) bp 94-98°C.

20

25

1,1-Dimethylethyl(S)-4-Formyl-2,2-Dimethyl-3-Oxazolidinecarboxylate (26)

30

The ester (25) (27.70g, 0.106mol) was dissolved in dry toluene (206ml) under an atmosphere of nitrogen and cooled to -78°C with stirring. A solution of DIBAL (120ml of a 1.5M solution in toluene, 0.18mol) was added over a 1 hour period with care being taken to prevent the internal temperature to rise above -65°C. The reaction was then

35

- 26 -

5 allowed to stir for a further 2 hours at -78°C , then quenched with cold (-78°C) methanol and the resulting solution poured into ice cold 1N HCl. Ethyl acetate (500ml) was then added and the solid aluminium salts removed by filtration. The filtrate was extracted twice with ethyl acetate, and the organic layers combined, dried (MgSO_4), filtered and evaporated. The residue was then distilled at reduced pressure to give the title compound (26) as a colourless oil (16.65g, 68%) bp $102-104^{\circ}\text{C}$.

10

1,1-Dimethylethyl [R-(R*,S*)]-2,2-Dimethyl-4-(-Hydroxy-2-Hexadecynyl)-3-Oxazolidinecarboxylate (27)

(where in structure 27 n is 12)

15 A solution of pentadec-1-yne (5g, 0.024mol) in dry THF (130ml) was cooled to -20°C under nitrogen, and treated with a solution of butyllithium (13.75ml of a 1.6m solution in THF, 0.022mol) over ten minutes. The resulting solution was maintained at -20°C for two hours and then treated with
20 freshly distilled HMPT (6.44ml) followed by a solution of the aldehyde (26) (4.23g, 0.818mol) in THF (10ml). The resulting solution was then allowed to warm to room temperature over two hours, quenched with ammonium chloride solution and extracted three times with ether. The
25 combined ethereal layers were evaporated to dryness and the residue purified by chromatography on silica (50g). Elution with 10% ethyl acetate in petrol gave the title compound (27) as a clear oil (4.24g, 52%).

30

D-Erythro-Sphingosine (7)

(where in structure 7, n is 12 and A is the group $-\text{CH}=\text{CH}-$)

35 Ethylamine (300ml) under nitrogen was cooled to -78°C with stirring and treated with lithium metal (2.25g, 0.324mol) and the resulting blue colour allowed to develop over 30 minutes at this temperature. A solution of the

- 27 -

acetylene (27) (10.04g, 0.229mol) in THF (300ml) was cooled to -20°C and added over a ten minute period and the resulting solution maintained for 1 hour at -78°C and allowed to warm to room temperature overnight. Solid ammonium chloride (38.65g, 0.729mol) was then added (fizzing) and the volatiles removed in vacuo. The residue was partitioned between ether and water, the organic layer separated, washed with water, dried (MgSO₄), filtered and evaporated to leave D-erythro-sphingosine as an off-white solid (6.36g, 92%).

C₂₂ lineoyl ceramide 1 (17)

A solution containing the acid (42) and sphingosine (7) in methylene chloride at room temperature was treated with chloromethylpyridinium iodide and triethylamine. The resulting solution was stirred for one hour, then diluted with methylene chloride and washed with water three times, then dried, filtered and evaporated to leave C₂₂-lineoyl ceramide 1 (17).

CHARACTERISATION

Infra-Red Spectroscopy

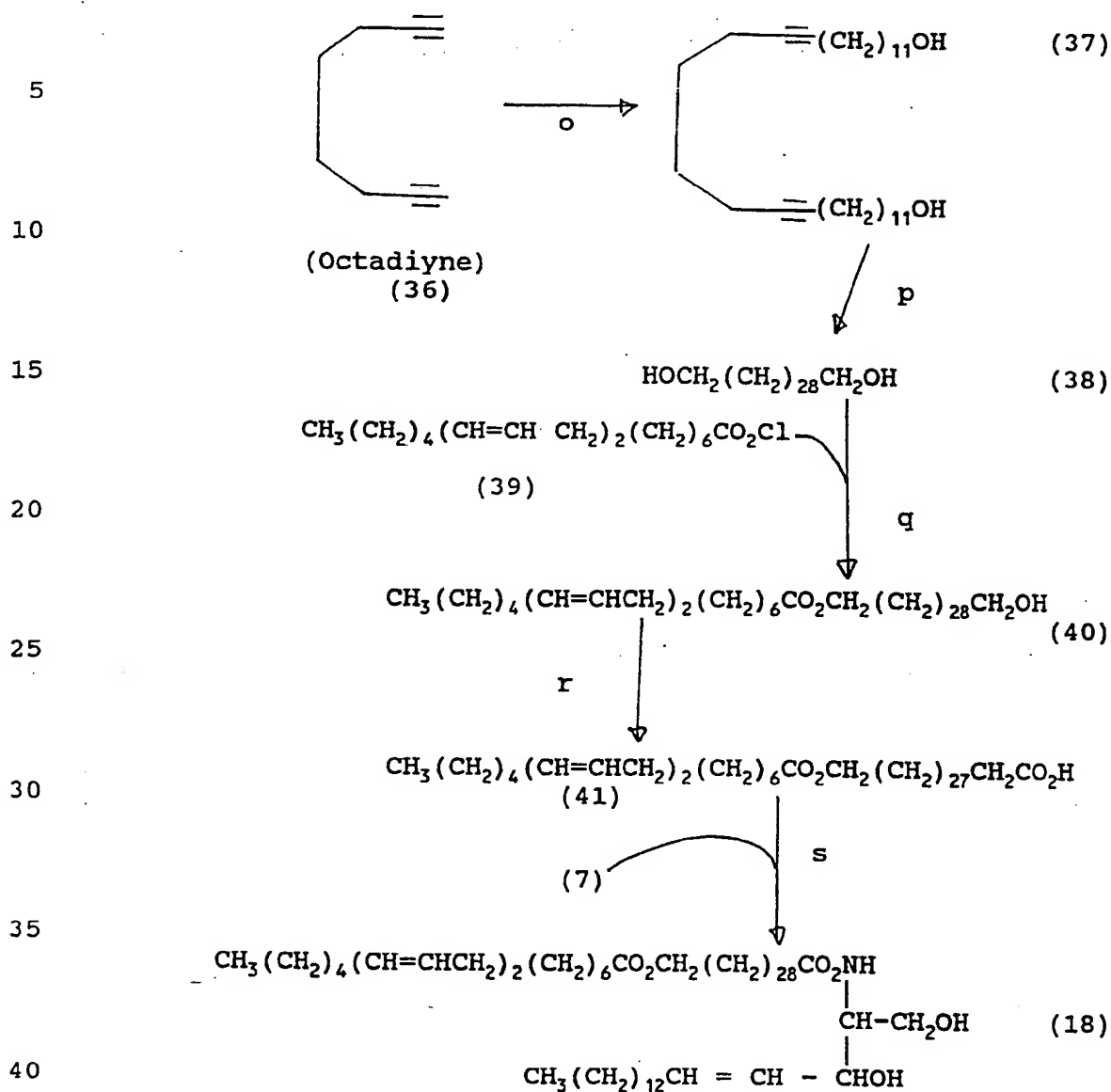
The sample of C₂₂ lineoyl ceramide 1 (17) was run as a cast film from CHCl₃ onto a KBr plate, on a Bruker IFS-88 FTIR spectrometer. The following features are fully consistent with the proposed structure for Ceramide 1.

30	alkyl -H	3000 - 2800 cm ⁻¹
	OH	3292 cm ⁻¹
	Ester C=O	1738 cm ⁻¹
	Amide C=O	~1643 cm ⁻¹
	C=C	~1643 cm ⁻¹
35	Amide N-H	1556 cm ⁻¹
	$\begin{array}{c} \text{H} \quad \quad \text{H} \\ \diagdown \quad \diagup \\ \text{C} = \text{C} \\ \diagup \quad \diagdown \\ \text{H} \quad \quad \text{H} \end{array}$	3011 cm ⁻¹

EXAMPLE 2Synthesis of C₃₀ Lineolyl Ceramide 1 (18)5 Synthesis of C₃₀ Linoleic Ceramide

The C₃₀ linoleoyl ceramide is the major component of ceramide one in human stratum corneum.

10 The C₃₀ ω-hydroxy fatty acid was synthesised from octadiyne and 11-bromoundecanol prior to synthesis of the 30-linoleoyloxytriacontanol by esterification with linoleoyl chloride. The acid is formed by oxidation with chromic acid before coupling to sphingosine with
15 chloromethylpyridinium iodide. Like the C₂₂ species, the major contaminant of the reaction product was the acyl acid. However, the chemistry presented here was more efficient giving a 80% purity. Ceramide one was purified by chromatography on aminopropyl bonded silica.

Scheme 3Reagents

- 45
- o Butyllithium, HMPA, 11-bromoundecanol, tetrahydrofuran (THF) then TsOH, MeOH
- p H₂, Pd, THF
- q Pyridine, CHCl₃
- r Chromic acid, ether
- s Chloromethylpyridinium iodide, triethylamine, CH₂Cl₂
- 50

- 30 -

Experimental for the Synthesis of C₃₀ Linoleic Ceramide 1.1,30-Dihydroxytriaconta-12,18-Diyne (37)

5 A solution of octadiyne (9.3ml; 0.07mol) in dry THF (200ml) at 0°C under nitrogen was treated with HMPA (24.38ml, 0.14 mol) and n-butyllithium (87.61ml of a 1.6M solution in hexane; 0.14mol). The resulting solution was left for ten minutes at 0°C and then treated with a solution of 11-
10 bromoundecanol (47g, 0.14mol). The resulting solution was allowed to stir at room temperature for four hours and then heated at reflux overnight, cooled, diluted with water and extracted with ether. The organic layer was washed with water twice, then dried, evaporated and the residue
15 dissolved in methanol (60ml). This solution was treated with a catalytic amount of toluenesulphonic acid and heated at reflux for ten minutes. On cooling the title compound (37) separated as pale yellow crystals (18.42g, 59%).

1,30-Triacontadiol (38)

A solution of 1,30-dihydroxytriaconta-12,18-diyne (11g, 0.024mol) in the THF (440ml) was maintained at room temperature under nitrogen and treated with 10% lead on
25 charcoal (1.1g). An atmosphere of hydrogen was introduced and the flask external temperature raised to 60°C. The reaction was allowed to proceed overnight, then filtered through celite and allowed to cool, which caused the product to separate as white crystals (10.67g, 95%).

30

30-Linoleoyloxytriacontanol (40)

1,30-Triacontadiol (38) (2.5g, 5.5mmol) was dissolved in dry, ethanol free chloroform at 60°C under nitrogen and
35 treated with pyridine (0.49ml; 6.05mmol). The resulting solution was treated with a solution of linoleoyl chloride (39) (1.64g; 5.5mmol) in chloroform (20ml) over a twenty

- 31 -

minute period and then maintained at 60°C for a further thirty minutes, then cooled, diluted with chloroform, washed with water three times, dried, filtered and evaporated. The crude product obtained in this manner was purified on silica (30g). Elution with chloroform gives the title compound (40) as a white solid (1.73g; 44%).

30-Linoleoyloxytriacontanoic Acid (41)

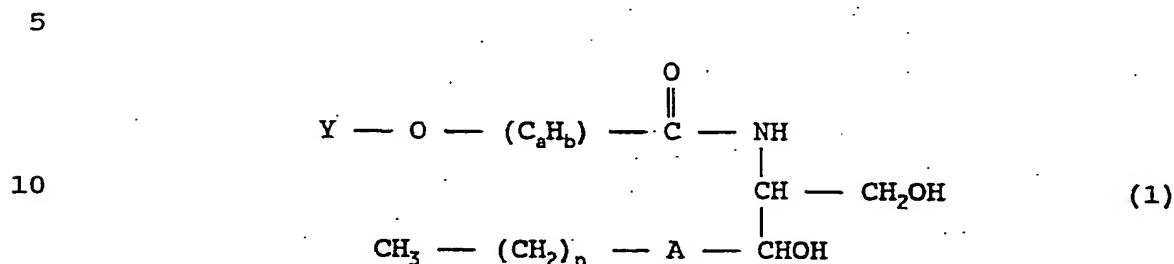
The alcohol (40) (1.73g, 2.41mmol) was dissolved in ether (200ml) at 30°C and treated with chromic acid (4ml of a 0.67M solution; 2.68mmol). The resulting solution was allowed to stir and cool to room temperature over two hours during which time two further 1ml portions of chromic acid were added. Propan-2-ol (5ml) was then added and the ethereal solution washed with water four times, then dried, filtered and evaporated to leave the title compound (41) as a white powder (1.57g; 89%).

C₃₀-Linoleic Ceramide (18)

A solution containing the acid (41) (1.57g, 2.15mmol) and sphingosine (0.643g, 2.15 mmol) in methylene chloride (175ml) at room temperature was treated with chloromethylpyridinium iodide, (0.548g, 2.15mmol) and triethylamine (0.6ml; 4.3mmol). The resulting solution was stirred for one hour, then diluted with methylene chloride (200ml) and washed with water three times, then dried, filtered and evaporated to leave C₃₀-linoleic ceramide (18) as a light-brown wax (2.17g, 98%).

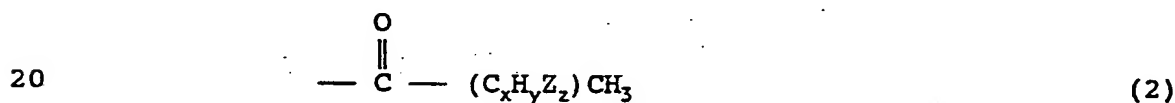
CLAIMS

1. A method of synthesising an ω -hydroxy fatty acid containing ceramide having the general structure (1)



where A represents CH_2 or $-\text{CH}=\text{CH}-$

Y represents a residue of a C_{14} to C_{22} fatty acid having the structure (2)



where Z is $-\text{OH}$ or an epoxy oxygen

x is an integer of from 12 to 20

y is an integer of from 20 to 40

z is 0 or an integer of from 1 to 4

a is an integer of from 8 to 50

b is an integer of from 10 to 100

and n is an integer of from 7 to 27

selected from synthesis A, synthesis B, synthesis C and synthesis D wherein;

synthesis A comprises;

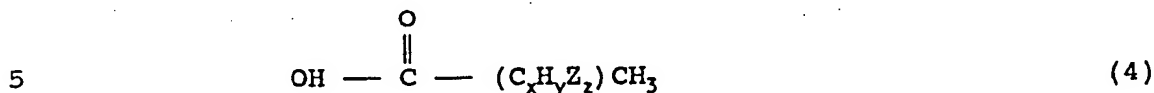
(ia) coupling an ω -hydroxy fatty acid with a protected carboxyl group having the general structure (3);



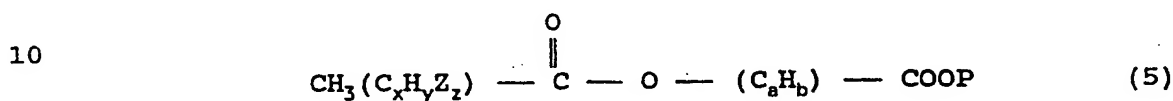
where P is a protection group

- 33 -

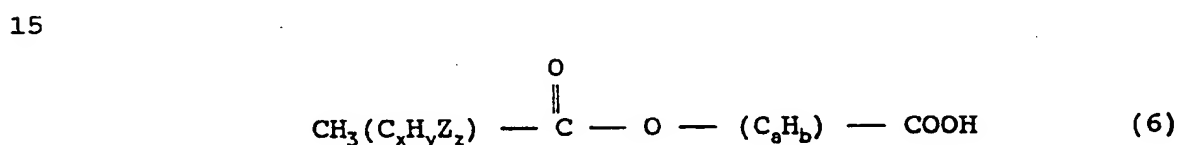
with a C₁₄₋₂₂ fatty acid having the general structure (4);



to give an intermediate having the general structure (5);

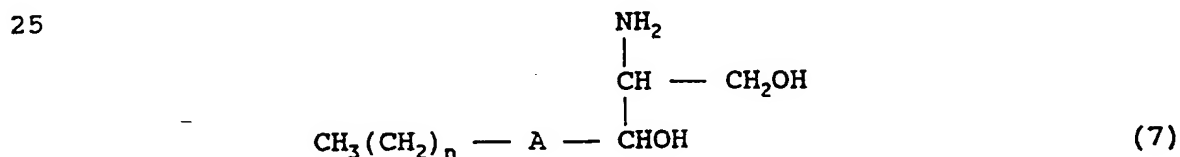


(iia) deprotection of the intermediate (5) to provide an intermediate having the general structure (6);



; and

(iia) coupling the intermediate (6) with sphingosine having the general structure (7);



to form the ω-hydroxy fatty acid containing ceramide having the general structure (1);

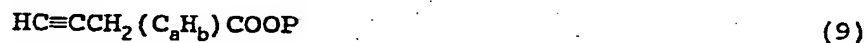
synthesis B comprises;

(ib) converting a terminal acetylenic alcohol having the general structure (8);



- 34 -

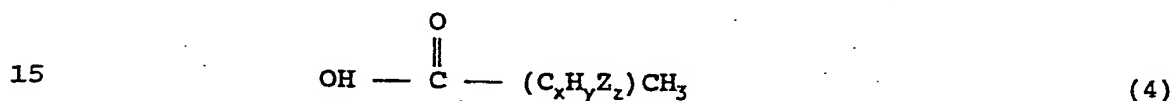
into an intermediate having the general structure (9);



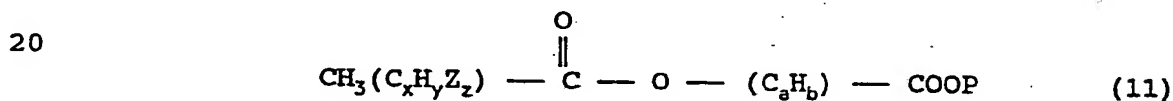
5 then converting this intermediate into an intermediate having the general structure (10);



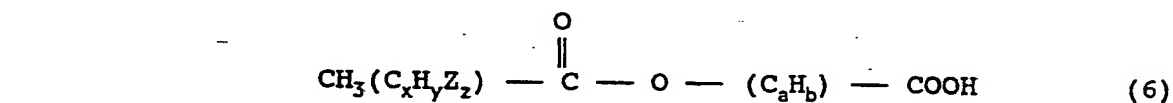
10 (iib) coupling the intermediate (10) with a C_{14-22} fatty acid having the general structure (4);



to give an intermediate having the general structure (11);

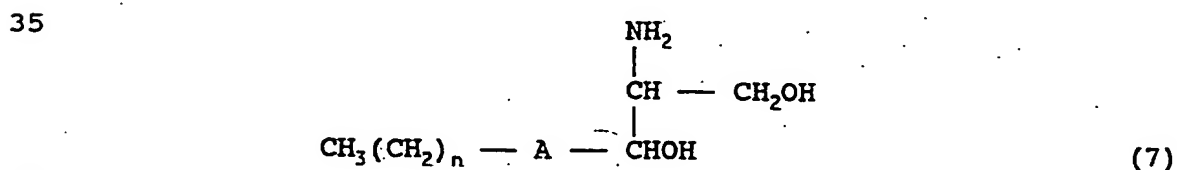


then selectively converting the intermediate (11) into an intermediate having the general structure (6);



30 ; and

(iiib) coupling the intermediate (6) with sphingosine having the general structure (7);



- 35 -

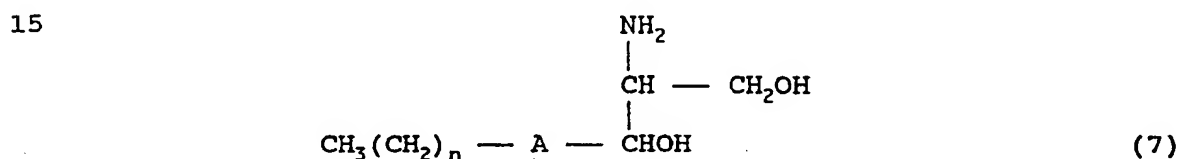
to form the ω -hydroxy fatty acid containing ceramide having the general structure (1);

synthesis C comprises:

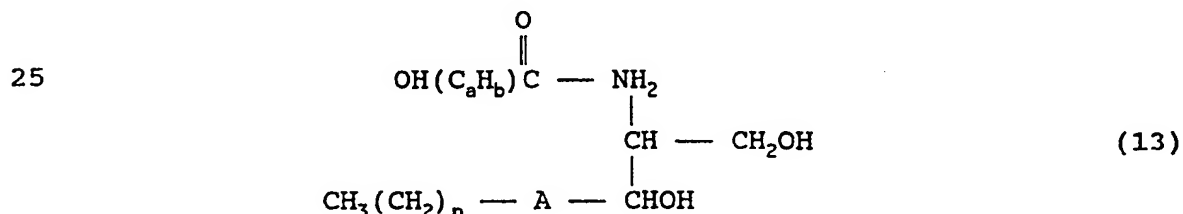
(ic) coupling an ω -hydroxy fatty acid having the general structure (12);



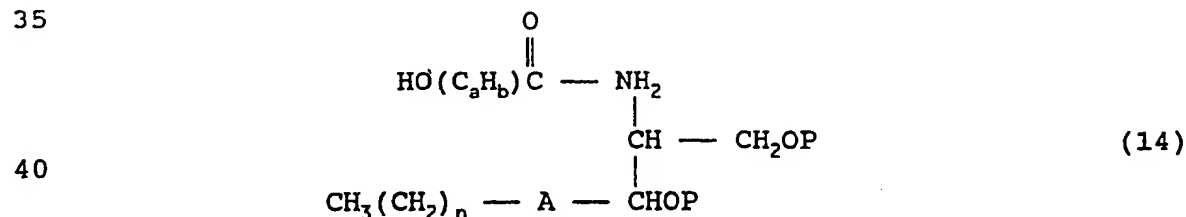
with a sphingosine having the general structure (7);



to form an intermediate having the general structure (13);



(iic) protection of the hydroxyl groups of the intermediate having the general structure (13) to give an intermediate having the general structure (14);



(iiic) esterification of the intermediate (14) with a C_{14} to C_{22} fatty acid having the general structure (4);

- 36 -



5 ; and

(ivc) removal of the protection groups to provide the ω -hydroxy fatty acid containing ceramide having the general structure (1); and

10

synthesis D comprises:

(id) coupling a long chain diol having the general structure (15);

15



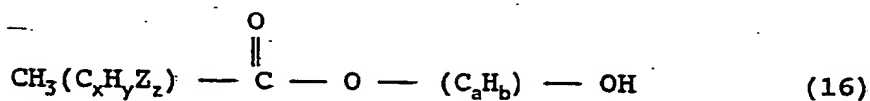
with a C_{14-22} fatty acid having the general structure (4);

20



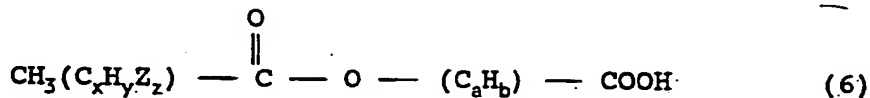
to give an intermediate having the general structure (16);

25



(iid) oxidation of the free hydroxyl group on intermediate (16) to give an intermediate having the general structure (6);

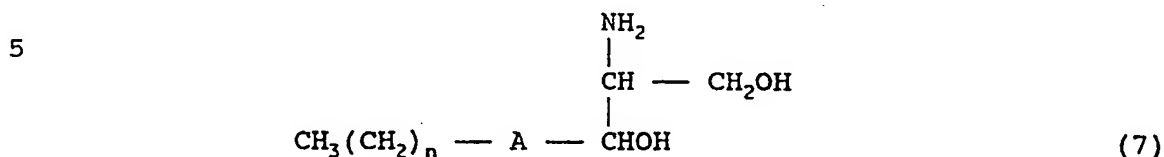
35



; and

- 37 -

(iiid) coupling the intermediate (6) with sphingosine having the general structure (7);



to form the ω -hydroxy fatty acid containing ceramide having the general structure (1).

2. A method of synthesising an ω -hydroxy fatty acid containing ceramide according to claim 1 comprising;

(ia) coupling a ω -hydroxy fatty acid with a protected carboxyl group having the general structure (3);

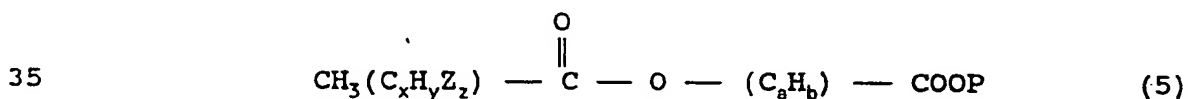


where P is a protection group
a is an integer of from 8 to 50; and
b is an integer of from 10 to 100

with a C_{14-22} fatty acid having the general structure (4);



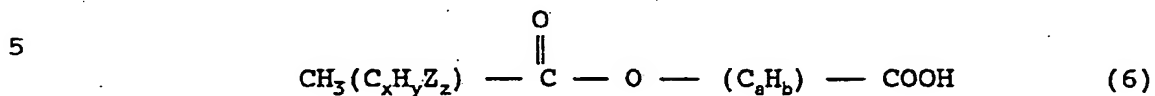
to give an intermediate having the general structure (5);



where x is an integer of from 12 to 20
y is an integer of from 20 to 40
z is 0 or an integer of from 1 to 4; and
Z is -OH or an epoxy oxygen.

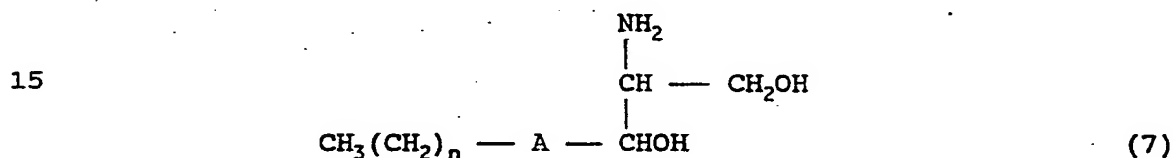
- 38 -

(iia) deprotection of the intermediate (5) to provide an intermediate having the general structure (6);



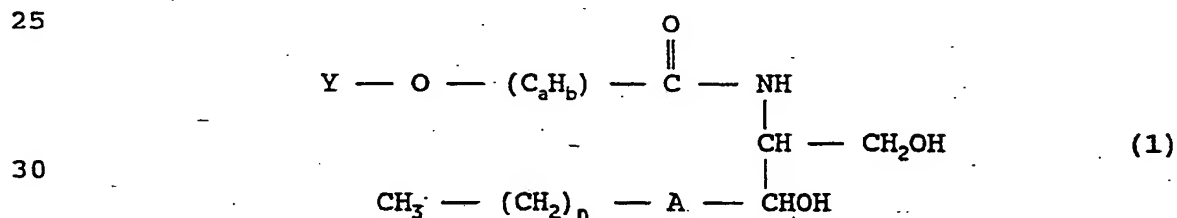
; and

(iia) coupling the intermediate (6) with sphingosine having the general structure (7);



where A represents CH_2 or $-\text{CH}=\text{CH}-$; and
n is an integer of from 7 to 27

to form the ω -hydroxy fatty acid containing ceramide having the general structure (1);



where Y represents a residue of C_{14} to C_{22} fatty acid having the structure (2).

35



- 39 -

3. A method of synthesising an ω -hydroxy fatty acid containing ceramide according to claim 1 comprising;

(ib) converting a terminal acetylenic alcohol having the general structure (8);



where a is an integer of from 8 to 50; and

b is an integer of from 10 to 100

into an intermediate having the general structure (9);



where P is a protection group

then converting this intermediate into an intermediate having the general structure (10);



(iib) coupling the intermediate (10) with a C_{14-22} fatty acid having the general structure (4);



where Z is -OH or an epoxy oxygen

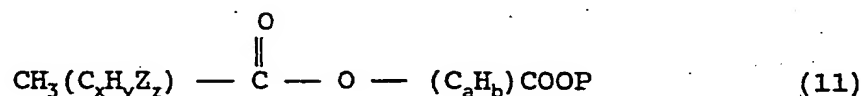
x is an integer of from 12 to 20

y is an integer of from 20 to 40; and

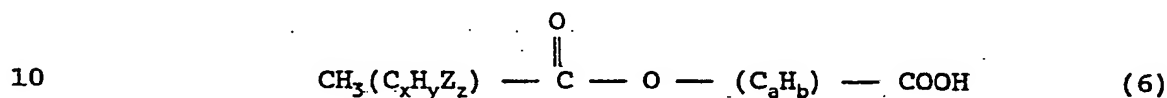
z is 0 or an integer of from 1 to 4

to give an intermediate having the general structure (11);

- 40 -

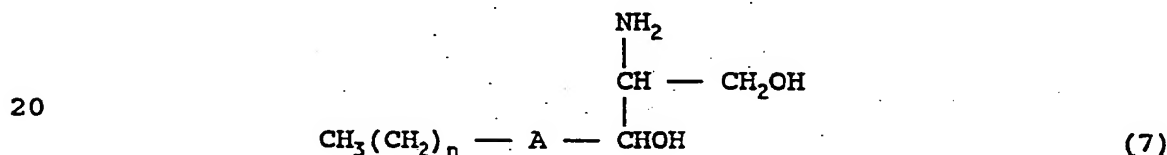


5 then selectively converting the intermediate (11) into an intermediate having the general structure (6);



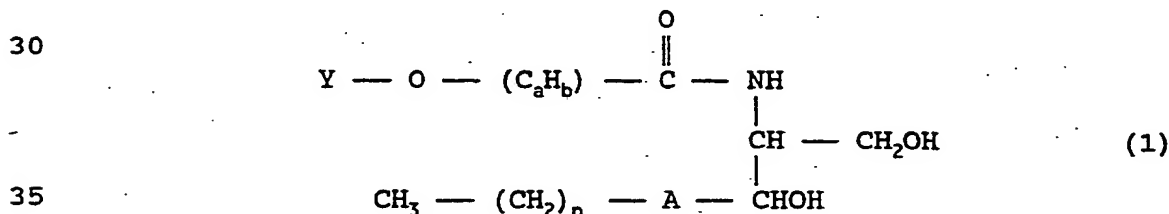
; and

(iiib) coupling the intermediate (6) with sphingosine
15 having the general structure (7);

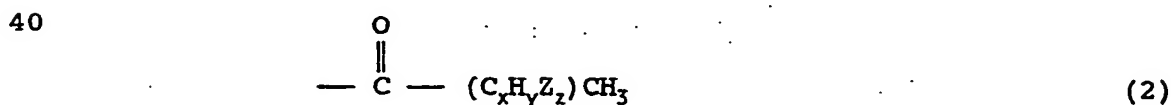


where A represents CH_2 or $-\text{CH}=\text{CH}-$; and
n is an integer of from 7 to 27;

25 to form the ω -hydroxy fatty acid containing ceramide having the general structure (1);



where Y represents a residue of a C_{14} to C_{22} fatty acid having the structure (2).



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4. A method of synthesising an ω -hydroxy fatty acid containing ceramide according to claim 1 comprising;

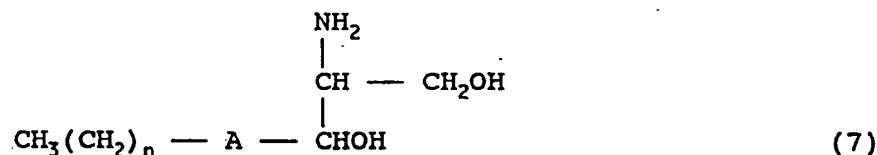
(ic) coupling an ω -hydroxy fatty acid having the general structure (12);



where a is an integer of from 8 to 50; and

b is an integer of from 10 to 100

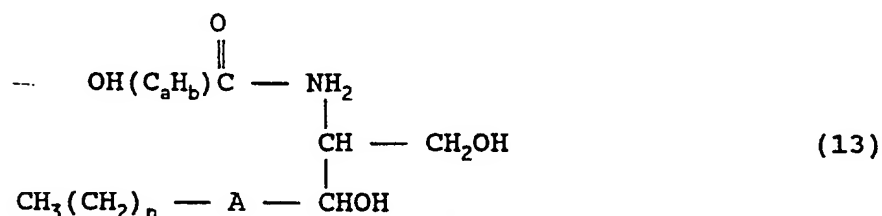
with a sphingosine having the general structure (7);



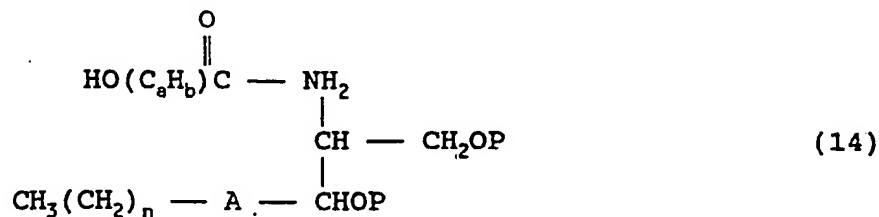
where A represents CH_2 or $-\text{CH}=\text{CH}-$; and

n is an integer of from 7 to 27;

to form an intermediate having the general structure (13);



(iic) protection of the hydroxyl groups of the intermediate having the general structure (13) to give an intermediate having the general structure (14);



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where P is a protection group;

(iiic) esterification of the intermediate (14) with a C₁₄ to C₂₂ fatty acid having the general structure (4):



where Z is -OH or an epoxy oxygen

x is an integer of from 12 to 20

y is an integer of from 20 to 40; and

z is 0 or an integer of from 10 to 100

; and

(ivc) removal of the protection groups to provide the ω-hydroxy fatty acid containing ceramide having the general structure (1).

5. A method of synthesising an ω-hydroxy fatty acid containing ceramide according to claim 1 comprising;

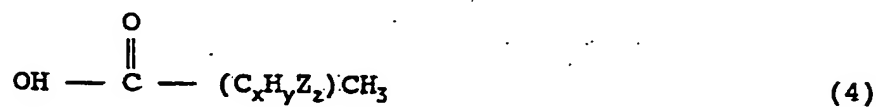
(id) coupling a long chain diol having the general structure (15);



where a is an integer of from 8 to 50; and

b is an integer of from 10 to 100

with a C₁₄₋₂₂ fatty acid having the general structure (4);



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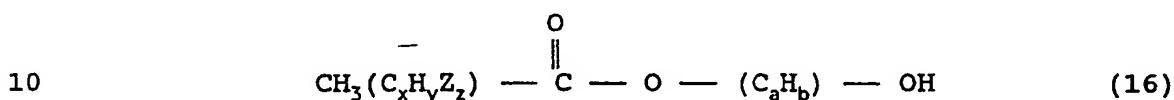
where Z is -OH or an epoxy oxygen

x is an integer of from 12 to 20

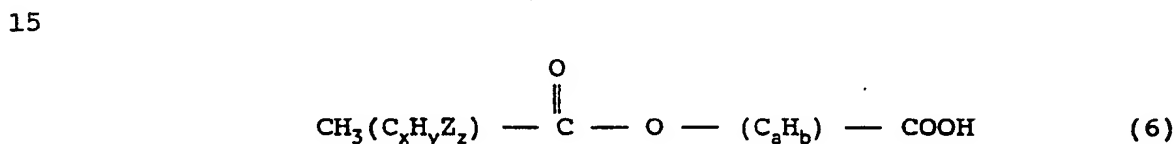
y is an integer of from 20 to 40; and

z is 0 or an integer of from 1 to 4

to give an intermediate having the general structure (16);

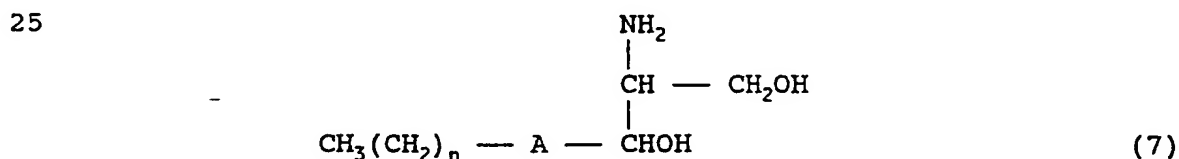


(iid) oxidation of the free hydroxyl group on intermediate (16) to give an intermediate having the general structure (6);



; and

(iiid) coupling the intermediate (6) with sphingosine having the general structure (7);



where A represents CH_2 or $-\text{CH}=\text{CH}-$; and

n is an integer of from 7 to 27

to form the ω -hydroxy fatty acid containing ceramide having the general structure (1).

6. A method of synthesising an ω -hydroxy fatty acid containing ceramide according to claim 1, claim 2, claim 3 or claim 4 wherein the protection group (P) is t-butylalcohol.

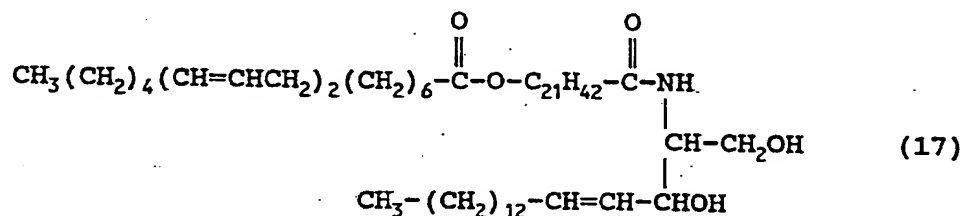
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7. A method of synthesising an ω -hydroxy fatty acid containing ceramide according to any preceding claim wherein the C_{14-22} fatty acid having the general structure (4) is linoleic acid.

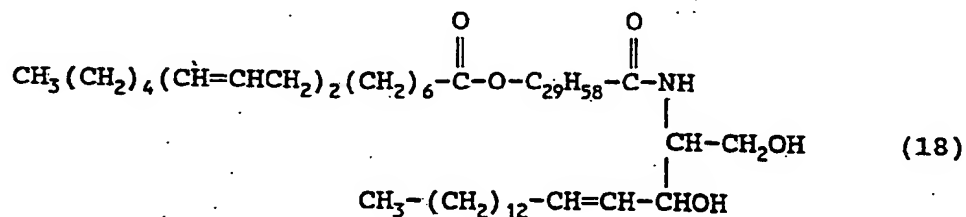
8. A method of synthesising an ω -hydroxy fatty acid containing ceramide according to any preceding claim wherein, in the sphingosine molecule having the general structure (7) n is 12.

9. A method of synthesising an ω -hydroxy fatty acid containing ceramide according to any preceding claim wherein in the sphingosine molecule having the general structure (7), A is the group $-\text{CH}=\text{CH}-$.

10. A method of synthesising an ω -hydroxy fatty acid containing ceramide according to any preceding claim wherein the ceramide synthesised has the structure (17).



11. A method of synthesising an ω -hydroxy fatty acid containing ceramide according to any preceding claim, wherein the ceramide synthesised has the structure (18).



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12. The use of a ceramide or mixture of ceramides synthesised by the method according to claim 1, within cosmetic compositions intended for topical application to human skin, hair or nails.

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13. A cosmetic composition comprising 0.0001 to 10% by weight of a ceramide or mixture of ceramides synthesised according to claim 1.

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14. A cosmetic composition according to claim 13, comprising 0.001 to 5% by weight of a ceramide or mixture of ceramides synthesised according to claim 1.

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 93/00932

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁵ : C 07 C 235/08, C 07 C 231/00, A 61 K 7/48		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC ⁵	C 07 C 235/00, C 07 C 231/00	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched *		
III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	EP, A2, 0 097 059 (UNILEVER NV) 28 December 1983 (28.12.83), pages 41-52; abstract (cited in the application). --	1, 7-9, 12-14
A	THE JOURNAL OF ORGANIC CHEMISTRY, vol. 53, issued 1988, P. GARNER et al. "A stereodivergent synthesis of d-erythro-sphingosine and d-threo-sphingosine from l-serine" pages 4395-4398 (cited in the application). --	1
A	EP, A1, 0 398 272 (KAO CORPORATION) 22 November 1990 (22.11.90), claims 2, 3. --	1, 12- 14
A	EP, A1, 0 482 860 --	1, 7,
<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>* Special categories of cited documents: ¹⁴</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Z" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search <div style="text-align: center;">23 July 1993</div>		Date of Mailing of this International Search Report <div style="text-align: center;">17. 08. 93</div>
International Searching Authority <div style="text-align: center;">EUROPEAN PATENT OFFICE</div>		Signature of Authorized Officer <div style="text-align: center;">KÖRBER e.h.</div>

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	(UNILEVER PLC) 29 April 1992 (29.04.92), claims.	12-14
	EP, A1, 0 293 006 (MECT CORPORATION) 30 November 1988 (30.11.88), pages 6-9.	1

ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/GB 93/00932 SAE 73493

In diesem Anhang sind die Mitglieder
der Patentfamilien der im obenge-
nannten internationalen Recherchenbericht
angeführten Patentdokumente angegeben.
Diese Angaben dienen nur zur Unter-
richtung und erfolgen ohne Gewähr.

This Annex lists the patent family
members relating to the patent documents
cited in the above-mentioned inter-
national search report. The Office is
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of information.

La présente annexe indique les
membres de la famille de brevets
relatifs aux documents de brevets cités
dans le rapport de recherche inter-
national visée ci-dessus. Les renseigne-
ments fournis sont donnés à titre indica-
tif et n'engagent pas la responsabilité
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Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A2 97059	28-12-83	AT E 47517	15-11-89
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